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13. ABSTRACT *(Maximum 200 words)*

Angiogenesis is vital to tumor growth and metastasis. The scope of this grant was to study in detail the role of Tissue transglutaminase (tTG) during wound healing and tumorigenesis. In the first year of the grant proposal, we have described the expression, localization, molecular form and tTG's association with other major determinants of wound healing and tumorigenesis. Our findings clearly show that tTG is readily upregulated in wound healing and rat mammary adenocarcinoma and is associated with endothelial and inflammatory cells. Hypoxia, Vascular endothelial growth factor, Transforming growth factor beta and Tumor necrosisi factor alpha are also upregulated alongside tTG in those cells. tTG is quickly proteolysed in the tissues and that may have important consequences as tTG can hydrolyze ATP/GTP in its fragmented form. We are submitting these findings to Journal of Clinical Investigation , Proceedings of National Academy of Sciences and American Journal of Pathology where we have detailed the potential significance of these observations. We have also addressed most of the tasks detailed in the statement of work and are now set to proceed with the rest of the project.

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INTRODUCTION:

Tissue Transglutaminase (tTG) is a member of family of important enzymes called Transglutaminases. These are calcium dependent enzymes which catalyze intermolecular covalent bonds. This crosslinking makes the proteins resistant to mechanical and proteolytic degradation. The purpose of our grant proposal was to describe the role of this extracellular matrix enzyme during wound healing and tumorigenesis as both processes are very similar in mechanism.

The importance of tTG in wound healing was initially based on the presence of defective wound healing in factor XIII deficiency. Bowness et al described the presence of tTG in wound healing in rats and made the important observation that although antigen and activity both increased, there was more antigen than activity indicating degradation or inactivation of the protein [1]. Application of putrescine, a transglutaminase inhibitor, produced a significant decrease in wound strength between day 5 and 10 [2].

tTG's involvement in tumor biology has been demonstrated by numerous studies. An inverse relationship between tTG activity and metastatic potential has been reported by several investigators in cells of murine origin [3,4]. Kinght et el reported on direct relationship between tTG activity and detergent insoluble apoptotic body formation in a number of metastatic cell lines cloned from a hamster fibrosarcoma [5]. Johnson et el transfected human tTG cDNA hamster sarcoma Met B cells and reported that overexpression of tTG lead to delay and suppression of tumor cell growth [6].

In addition, tTG has been implicated in apoptosis, cellular signaling, bone formation and its list of substrates is extensive and includes all major extracellualr proteins. Thus, it is vital to understand the basic mechanisms regarding the working of this enzyme to effectively use it for novel therapeutics for breast cancer.

BODY:

We realized the need of first describing the expression, molecular form of tTG and its association with other major modulators of wound healing such as Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor beta (TGF beta), Tumor Necrosis Factor alpha (TNF alpha) and Hypoxia. This would clearly help establish tTG's role during wound healing and tumorigenesis and also suggest possible mechanisms to its role during these processes.

We are now in the process of submitting this information as three publications (see appendix) which detail our findings in this regard. We have found that tTG is expressed by endothelial cells, macropahges and fibroblasts

during both wound healing and R3230 Mammary adenocarcinoma. tTG expression is most closely related to TGF beta. tTG is also rapidly degraded and is present in 50-55 kda and 20 kda form. We will forward the final accepted versions of the papers later.

We have also addressed the major tasks to be completed during the first year. We will detail them task by task:

Task 1 and 3: We have isolated fibrinogen from rats and prepared for the experiments outlined in the grant. We have also ran comparisons of that fibrin with the commercially available fibrin from Sigma and found that our isolation procedure deactivates native Factor XIIIa, which results in weak fibrin gels. We will supplement the gels with 5ug/ml of Factor XIIIa for the experiments.

We also have expressed recombinant tTG and its mutants in a E. Coli based expression system. Our yield of the protein was low in earlier expressions and we had to repeat the procedure to have enough quantity of the proteins for future experiments.

Both these processes took a little more time than expected which pushed our time line on other tasks.

Task 5 and 6: We started by manufacturing our own chambers but we could not reduce the size of the chamber down from 3 mm in width. We have now moved on with Millipore chambers and modified them for our use. The width is down to 1.5 mm which will save a lot of precious proteins and time from now on.

We are in the process of doing fibrin gel experiments to assess the dose curve of tTG in the fibrin chamber. We will relate the data in next report.

Task 2: We are setting up fibrinolysis assays to assess the tTG's ability to impart more ability to the fibrin gel and make it more resistant to proteolysis against plasmin. In this regard, I am also collaborating with other investigators in using a rat aorta model developed by Nicosia to better answer this question.

Task 4: We have not been able to address this aim in the last year due to overruns by other tasks. we have started work on this aim and are In the process of establishing the required assays to accomplish this task.

CONCLUSIONS:

Our work has established tTG as one of the prominent players during wound healing and tumor development and also how it is associated with other prominent cytokines and cells during these processes. Its ability to enhance angiogenesis during wound healing and limit tumor growth gave interesting insights into its activities. This sets up nicely for our work with fibrin gel experiments which would define the mechanisms of its effects during wound healing and tumor development.

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List Of Publications/Abstracts where Defense Grant was Cited:

1. **ZA Haroon,** KG Peters, CS Greenberg and MW Dewhirst. Ch:1 Angiogenesis and oxygen transport in the solid tumors in *Antiangiogenic Agents* (BA Teicher, ed) Humana, Totowa, NJ (In press).
2. **ZA Haroon**, JM Hettasch, TS Lai, RL McCauly, MW Dewhirst and CS Greenberg. Tissue Transglutaminase expression during rat dermal wound healing and it can function as a pro-angiogenic molecule (Being submitted to Journal Of Clinical Investigation)
3. **ZA Haroon**, JA Raleigh, CS Greenberg and MW Dewhirst. Interrelationship between Hypoxia, Cytokines and Inflammatory cells during rat dermal wound healing. (Being submitted to Proceedings of National Academy of Sciences)
4. **ZA Haroon**, R Syal, RL McCauly, RA Lindberg, JM Hettasch, MW Dewhirst and CS Greenberg. Tissue Transglutaminase blocks growth of R3230 Mammary Adenocarcinoma in Rat window chamber (Being submitted to American Journal Of Pathology).
5. MR Matthews, **ZA Haroon**, AH Friedman, CJ Wikstrand, CS Greenberg, JL Burchette, DD Bigner, RE Mclendon. Extracellular Matrix Antigens in Capillary Hemangioblastoma: an Immunohistochemical Study. (Being submitted to Journal of Histochemistry and Cytochemistry).

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